American Journal of Hematology 62:159–164 (1999)

Altered Expression of CD45 Isoforms in Differentiation of Acute Myeloid Leukemia

Hayato Miyachi,[1]* Yumiko Tanaka,[2] Kazumi Gondo,[2] Tsutomu Kawada,[2] Shunichi Kato,[3] Tamotsu Sasao,[4] Tomomitsu Hotta,[4] Shuichi Oshima,[1] and Yasuhiko Ando[1]

[1]Department of Clinical Pathology, Tokai University School of Medicine, Isehara, Japan
[2]Central Clinical Laboratory, Tokai University Hospital, Isehara, Japan
[3]Department of Pediatrics, Tokai University School of Medicine, Isehara, Japan
[4]Department of Internal Medicine, Tokai University School of Medicine, Isehara, Japan

Specific expression of different CD45 isoforms can be seen in various stages of differentiation of normal nucleated hematopoietic cells. Association of membrane expression of CD45 isoforms and differential levels of leukemia cells was studied in 91 cases with *de novo* acute myeloid leukemia (AML). Membrane expression of CD45RA and CD45RO was analyzed by flow cytometry and their expression patterns were compared with AML subtypes classified according to the French–American–British (FAB) classification. CD45RA was essentially expressed in all of the FAB myelocytic subtypes (M0–M3). Its expression in percentage was lower in the most differentiated subtype of AML (M3) when compared with other myelocytic subtypes. CD45RO expression was rarely observed in cases with myelocytic subtypes (1/56 cases of M0, M1, M2, and M3) except for the minimally differentiated myelocytic subtype (M0) or those with potential for differentiation to T-cell lineage where three of 12 cases showed CD45RO expression. When leukemia cells of an M3 case were differentiated to mature granulocytes by treatment of *all-trans*-retinoic acid, they showed increasing expression of CD45RO. In subtypes with a monocytic component (M4 and M5), both of CD45RA and CD45RO expression were observed and mutually exclusive. When 10 cases of M5 were subdivided by the differential level into undifferentiated (M5a) and differentiated monocytic leukemia (M5b), expression of CD45RA and CD45RO was strictly restricted to cases with M5a and M5b, respectively. These results suggest that CD45 isoform expression in AML characterizes differential levels both in myelocytic and monocytic lineages and specifically disturbed in each subtype. The assessment of CD45 isoform expression appears to provide an insight on biological characteristics and a useful supplementary test for differential diagnosis of AML subtypes. Am. J. Hematol. 62:159–164, 1999. © 1999 Wiley-Liss, Inc.

Key words: CD45 RA; CD45 RO; acute myeloid leukemia

INTRODUCTION

The CD45 proteins constitute a family of cell surface glycoproteins and are expressed in most of nucleated hematopoietic cells [1]. The CD45 family is generated through the alternative splicing of three exons out of 34 in CD45 gene. Exons 4, 5, and 6 are used differentially to generate as many as eight different mRNAs, and at least four isoform proteins of various molecular masses ranging from 180- to 220-kDa are known [2–5]. The 220- and the 180-kDa isoforms are designated as CD45RA and CD45RO, respectively.

CD45 molecules have tyrosine phosphatase activity in the cytoplasmic domain and are thus associated with protein phosphorylation and signal transduction [6,7]. The control of the exon usage results in a specific expression of different isoforms in the various stages of differentiation in most of nucleated hematopoietic cells. The expression of CD45RA and CD45RO in normal T-cell, B-cell, and myeloid cell subsets has been reported to be associated with the degree of differentiation in the sense that T-cell maturation is usually characterized by a switch from CD45RA+ to CD45RO+ [8–10]. The

*Correspondence to: H. Miyachi, Department of Clinical Pathology, Tokai University School of Medicine, Bouseidai, Isehara, Japan. E-mail: miyachi@is.icc.u-tokai.ac.jp

Received for publication 18 December 1998; Accepted 4 August 1999

CD45RA+ subset T-cells is virgin with respect to antigen exposure, whereas CD45RO+ T-cells seem to be antigen-educated memory cells [8,9]. Upon activation in vitro, CD45RA+ T-cells lose the CD45RA and acquire the CD45RO antigen. B-cells usually express CD45RA antigen, but a transition of CD45RA+ to the CD45RO+ occur at the terminal stages of differentiation [10]. In normal myelopoiesis, committed progenitors of myeloid lineage are composed of CD45RA+ cells, and CD45RO first appears at blast/promyelocyte stage and increase consistently during later differentiation [10,11]. Thus, most human macrophages and granulocytes express CD45RO antigen [11]. Expression of CD45 isoform RA/RO has been studied in myelocytic leukemia [11–14] as well as in lymphocytic leukemia [15–17]. In acute myeloid leukemia (AML), prevalent CD45RA expression has been suggested to relate to the ability of maintenance of abnormal proliferation by the malignant myeloid progenitor cells [11,12]. However, significance of CD45 isoforms in AML cell differentiation in each cell lineage is not well established. In order to clarify the significance of the CD45 isoforms in AML, the relationships between the expression of CD45 isoform RA/RO and differential levels in AML cells was studied in a series of untreated de novo AML. It was suggested that expression of CD45 isoform RA/RO in AML was associated with differentiation both in myelocytic and monocytic lineages and specifically disturbed in each subtype of the French–American–British (FAB) classification. This study support the biological relevance of the FAB classification which is principally based on morphological criteria [18].

MATERIALS AND METHODS

Patients

Included in this study was a total of 91 patients who was diagnosed as having de novo AML during September 1992 and May 1998 at Tokai University Hospital, ages ranging from 9 months to 94 years. In the performance of the study, they were classified according to the FAB classification [18,19] into M0 ($n = 12$), M1 ($n = 27$), M2 ($n = 17$), M3 ($n = 12$), M4 ($n = 13$), and M5 ($n = 10$). M6 cases were excluded from the study.

Immunophenotyping

Immunophenotyping was performed on bone marrow samples, using standard methods with some modifications [20]. In brief, fresh marrow cells were treated with 1× red blood cell lysing solution (150 mM NH_4Cl, l0 mM $KHCO_3$, 0.1 mM EDTA, pH 7.3). Following a 30-min incubation at 37°C, the cells were washed with phosphate-buffered saline solution and stained by direct immunofluorescence with a panel of monoclonal antibodies. The mononuclear cell fraction in all evaluable cases contained at least 70% morphologically malignant cells. Attempts were made to gate primarily on the subpopulation of malignant cells. Cells were analyzed using a FACScan (Becton Dickinson, Mountain View, CA) flow cytometer. Each measurement contained at least 10,000 events. The monoclonal antibodies CD45RA (Leu18) and CD45RO (Leu-45RO) were obtained from Becton Dickinson (San Jose, CA). Background staining was detected using nonspecific immunoglobulin G mouse antibody. Findings were expressed as percentage of positive cells.

A positive reaction was defined as 20% of gated cells being more fluorescent than the control. Biphenotypic leukemias were defined according to the criteria described by Catovsky [21].

RESULTS

Expression of CD45 Isoforms in Acute Myeloid Leukemia

Figure 1 shows expression patterns of CD45 isoforms in AML. Most of cases with myelocytic subtypes (M0–M3) essentially displayed CD45RA. In contrast, CD45RO expression was rarely observed in cases with myelocytic subtypes (1/56 cases of M0, M1, M2, and M3) except for the minimally differentiated myelocytic subtype (M0) or those with potential for differentiation to T-cell lineage where three of 12 cases showed CD45RO expression (Table I). In all of them, blasts were peroxidase-negative and at least one of myeloid-associated marker (CD13 or CD33) -positive. Additional markers for T-lymphoid antigens were positive: all of them were CD7-positive and 4 of them were CD2-positive. Three of the 4 cases with CD2 expression showed CD45RO expression. When these cases with CD2 expression were considered as biphenotypic leukemias [21,22] and excluded from the M0 subtype, CD45RO expression was rarely observed in cases with all the myelocytic subtypes including M0. The percentage of positive cells for CD45RA antigen was lower in the most differentiated subtype of AML (M3) when compared with other myelocytic subtypes (Table II).

Expression of CD45 Isoforms in Acute Myeloid Leukemia Subtypes With a Monocytic Component

In FAB subtypes with a monocytic component (M4 and M5), both of CD45RA and CD45RO expression were observed. The two antigens showed a trend of mutual exclusion (Fig. 1). The results of Spearman's test showed a significant inverse relationship between the two antigens ($R = -0.61$, $P < 0.01$). When 10 cases of M5 were subdivided by the differential level into undifferentiated (M5a) and differentiated monocytic leukemia (M5b), expression of CD45RA and CD45RO was strictly restricted to cases with M5a and M5b, respectively

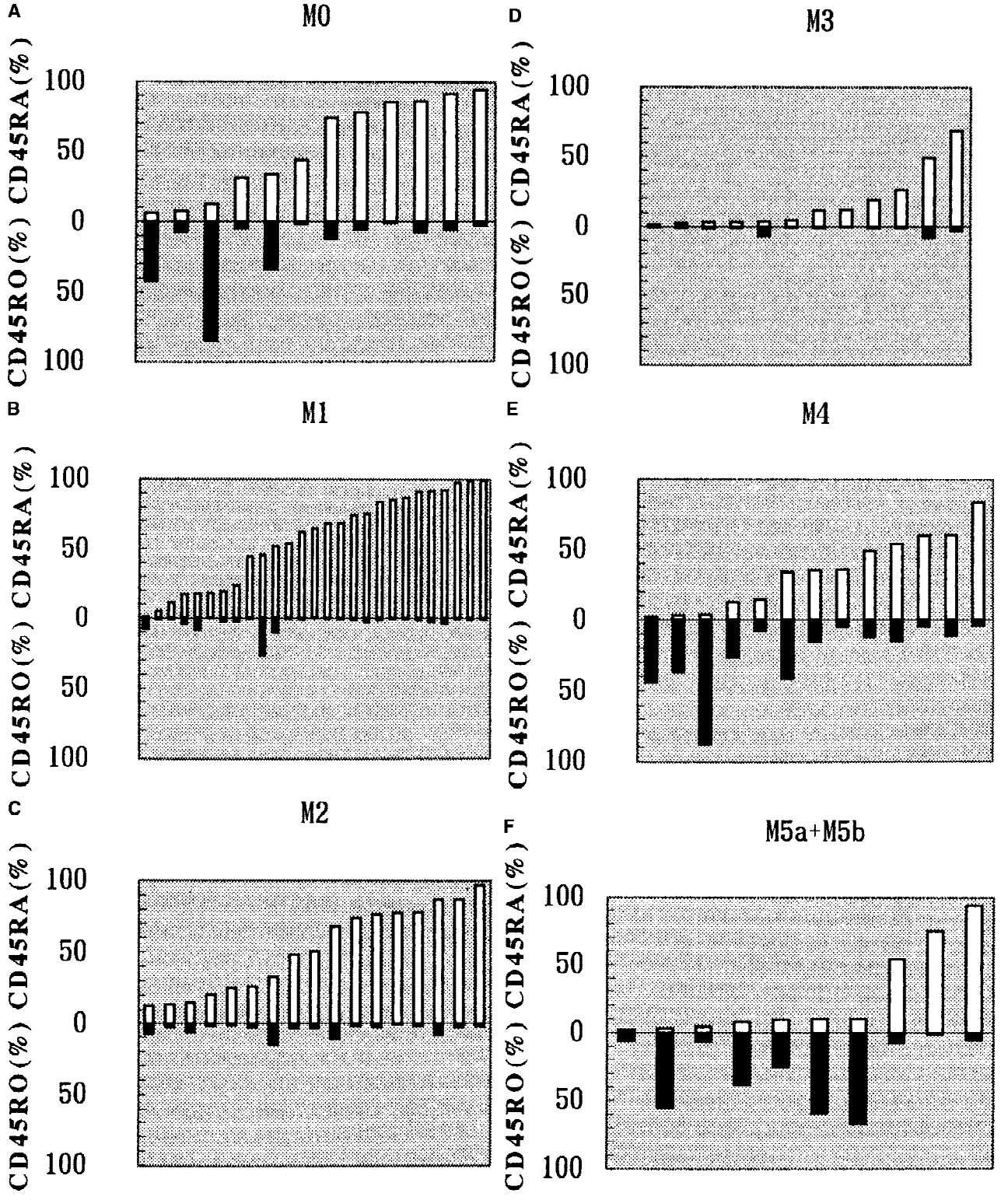

Fig. 1. Correlation between CD45RA and CD45RO expression in each subtype of acute myeloid leukemia. Paired values in each case of CD45RA (white bars) and CD45RO (black bars) are expressed as a percentage of positive cells. Increasing values of CD45RA are orderly plotted in the upper half of panels, whereas corresponding values of CD45RO for each case are shown in the lower half. (A) M0, (B) M1, (C) M2, (D) M3, (E) M4, (F) M5.

TABLE I. Expression of CD45 Isoforms in Acute Myeloid Leukemia*

	CD45 RA/RO patterns				
	RA^+RO^-	RA^-RO^+	RA^+RO^+	RA^-RO^-	Total
M0	8	2	1	1	12
M1	19	0	1	7	27
M2	14	0	0	3	17
M3	3	0	0	9	12
M4	7	4	1	1	13
M5	3	5	0	2	10
Total	54	11	3	23	91

*Expression of CD45 isoforms in acute myeloblastic leukemia was analyzed by flow cytometry. A positive reaction was defined as 20% of gated cells being more fluorescent than the control.

TABLE II. Positive Percentages for Expression of CD45 Isoforms in Acute Myeloid Leukemia*

	CD45 RA (%)	CD45 RO (%)
M0	53.5 ± 34.6	17.5 ± 24.9
M1	57.2 ± 32.5	3.3 ± 5.3
M2	52.1 ± 29.9	4.3 ± 4.0
M3	16.9 ± 21.4[a]	2.3 ± 2.7
M4	34.2 ± 26.1	23.9 ± 23.8
M5	28.8 ± 34.1	27.3 ± 25.7
M5a	45.7 ± 41.4	5.5 ± 2.4
M5b	8.0 ± 3.0	49.1 ± 17.0

*Percentages of positive cells for CD45RA or CD45RO in each FAB subtype were expressed as $m \pm$ SD.
[a]Significantly lower ($P < 0.01$) when compared with M0, M1, or M2.

(Figure 2, Table II). Three of five (60%) of M5a cases showed CD45RA, and all of them lacked CD45RO expression. All of five M5b cases showed CD45RO expression but not CD45RA. Staining profiles of CD45RA and CD45RO in representative M5a and M5b cases were shown in Figure 3.

Expression of CD45 Isoforms During Differentiation in Acute Promyelocytic Leukemia

A patient with acute promyelocytic leukemia (M3) whose leukemia cells did not display either CD45RA or CD45RO was treated with *all-trans*-retinoic acid. As leukemia cells of the patient were differentiated to mature granulocytes by treatment of *all-trans*-retinoic acid, they showed increasing expression of the CD45RO from 1.4% to 48.3% of positive cells (Fig. 4). Meanwhile they also showed minimal expression of CD45RA from 3.1% to 24.1% of positive cells in addition to expression of CD45RO.

DISCUSSION

Flow cytometrical analysis of membrane expression of CD45RA and CD45RO in cases with de novo AML demonstrated that M1, M2 and M3 cases had a trend to

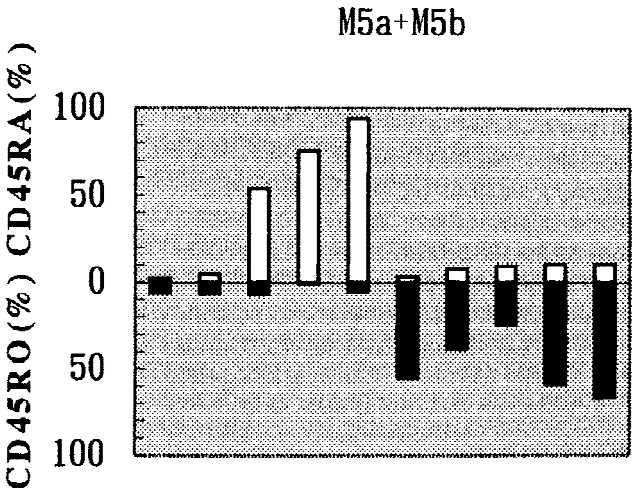

Fig. 2. Correlation between CD45RA and CD45RO expression in M5 subtypes of acute myeloid leukemia. Paired values in each case of CD45RA (white bars) and CD45RO (black bars) are expressed as a percentage of positive cells. Increasing values of CD45RA are plotted in order in the upper half of panels, whereas corresponding values of CD45RO for each case are shown in the lower half. Ten cases of M5 were subdivided by the differential level into undifferentiated (M5a) and differentiated monocytic leukemia (M5b). The paired values of CD45RA and CD45RO in five M5a and five M5b cases are plotted in the left and the right half of the panel, respectively.

express CD45RA without a concomitant increase in CD45RO. CD45RA expression was significantly decreased in the mature stage of myeloid leukemia (M3). Prevalently CD45RA+ cells comprise the majority of committed progenitors of myeloid lineage as well as T- and B-cells. Since normal myeloid cells do not express surface CD45RA, it can be inferred that CD45RA expression in M0, M1, M2, and M3 may be related to the ability of abnormally proliferating precursor cells to maintain an undifferentiated state, as has been suggested by other investigators [11,12].

In normal myelopoiesis, CD45RO first appears at blast/promyelocyte stage and increase consistently during later differentiation [10,11]. However, most cases with M3 displayed no expression of CD45RO, similar to other myelocytic subtypes. When the leukemia cells were differentiated to mature granulocytes by treatment of *all-trans*-retinoic acid, they showed increasing expression of CD45RO. This is in agreement with an in vitro study by Siavonne et al. who demonstrated that *all-trans*-retinoic acid promoted an up-regulation of CD45RO in parallel with a concomitant down-regulation of CD45RA in de novo AML cells including M3 promyelocytes [14]. These results suggest that CD45RO expression is associated with differentiation in myelocytic lineages, and its regulation appears disturbed in M3 cases and can be induced by the differentiating agents such as *all-trans*-retinoic acid. Interestingly, when the leukemia cells were

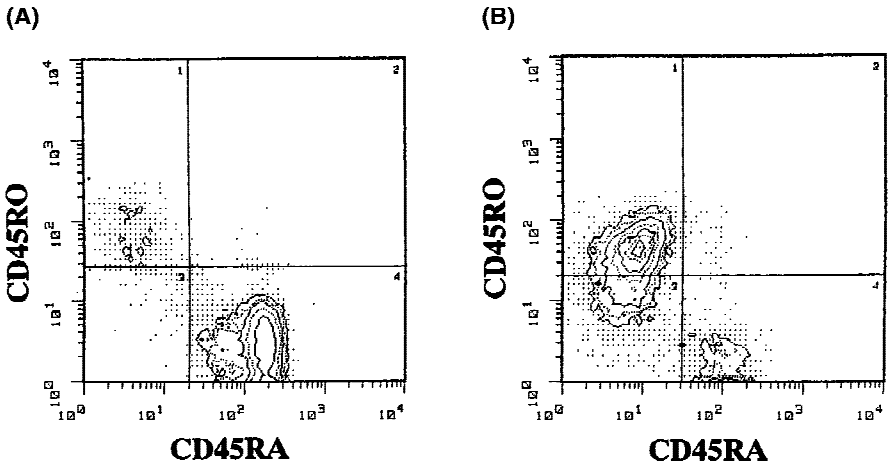

Fig. 3. Staining profiles of CD45RA and CD45RO in representative M5a and M5b cases. The most blasts in the M5a and M5b case express CD45RA and CD45RO, respectively. (A) M5a, (B) M5b.

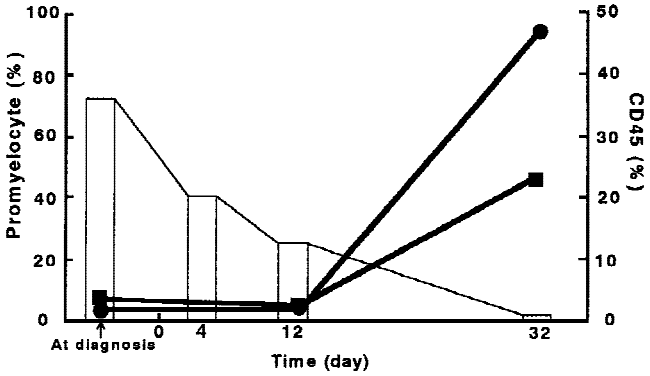

Fig. 4. Expression of CD45 isoforms in acute promyelocytic leukemia cells during differentiation by treatment of *all-trans*-retinoic acid. A patient with acute promyelocytic leukemia whose leukemia cells did not display either CD45RA or CD45RO was treated with *all-trans*-retinoic acid. Percentages of promyelocytes in all nucleated cells of bone marrow (white column) and positive percentages for CD45RA (●) or CD45RO (■) are shown. As the leukemia cells of the patient were differentiated to mature granulocytes by treatment of *all-trans*-retinoic acid, the CD45RO was increasingly expressed. In addition to CD45RO expression, CD45RA was also minimally expressed.

differentiated to mature granulocytes by treatment of *all-trans*-retinoic acid, they also showed minimal increase in expression of CD45 RA. It has been demonstrated that CD45RA antigen is present in the cytoplasm of mature granulocytes in spite of lack of its surface expression [11].

In contrast to myelocytic subtypes, those with a monocytic component had a trend to express both of CD45RA and CD45RO. CD45RO has been associated with monocytic differentiation in AML [13]. However, Master PS et al reported that blasts in M5 cases typically showed CD45RA+RO−. In the current study, when M5 cases were subdivided by the differential level into undifferentiated (M5a) and differentiated monocytic leukemia (M5b), expression of CD45RA and CD45RO was strictly restricted to cases with M5a and M5b, respectively. This is in agreement with a finding by Kawano et al. that CD45RO expression increased in monocytic blasts with more matured morphology [13]. In previous studies, M5 cases have not been subdivided by the differential level, probably because of small number of M5 cases. Since CD45RO and RA are coexpressed in most monocytes [12], predominance of CD45 isoforms in M5 cases indicated altered regulation of CD45 expression associated with neoplastic transformation. The isoform that is expressed is closely related to the differentiation stages of monoblasts. These results suggest that the regulation system which governs expression of CD45 isoforms in AML are disturbed in differentiation of AML cells and CD45RO expression is associated with differentiation in not only myelocytic but also monocytic lineages.

In contrast to other myelocytic subtypes, occasional AML cases with the earliest stage of myeloid differentiation showed CD45RO. CD34+ fraction of the normal bone marrow cells can be divided into CD45RA−RO+ early progenitors that give rise to long-term culture initiating cells and CD45RA+RO− late myeloid progenitor cells [23]. It can be inferred that CD45RO expression in cases with the earliest stage of myeloid differentiation may be related to its origin in the CD45RA−RO+ early progenitors. The putative malignant counterpart of pluripotent stem cells could be represented by a consistent subset of chronic myelogenous leukemia in blastic phase, in which blasts are CD45RO+ [24]. The AML cases with CD45RO expression had additional CD2 expression. CD2 is a marker for T-lymphoid antigens and unexpectedly seen in myeloblasts [21,22]. Since the majority of human thymocyte express high levels of CD45RO in the earliest stage of T-cell ontogenesis [24], it can also be inferred that CD45RO expression in the minimally differentiated myeloid leukemia cases may be related to their involvement of hematopoietic cells with potential for differentiation to T-cell lineage.

CD45 antigen molecules are supposed to regulate non-

receptor src-type tyrosine kinases through their phosphatase activity. The status of differentiation, activation, and proliferation in myeloid cells is supposed to determine which type of tyrosine kinase is in operation to select the expression pattern of CD45 isoforms. It is suggested that the regulation system which governs expression of CD45 isoforms in AML are disturbed and associated with state of abnormal differentiation and proliferation as well as lineage involvement. Each subtype of AML showed specific expression of different CD45 isoforms, reflecting that can be seen in the involved stages of differentiation of normal nucleated hematopoietic cells. The assessment of CD45 isoform expression appears to provide an insight on biological characteristics of blasts and a useful supplementary test for differential diagnosis of AML subtypes. This study supports the biological relevance of the FAB classification which is principally based on morphological features. Further studies are needed to clarify how the signal transduction is operated and associated with the abnormal differentiation and proliferation.

REFERENCES

1. Thomas ML. The leucocyte common antigen family. Annu Rev Immunol 1989;7:339–369.
2. Hall LR, Streuli M, Schlossman SF, Saito H. Complete exon–intron organization of the human leucocyte common antigen (CD45) gene. J Immunol 1988;141:2781–2787.
3. Johnson NA, Meyer CM, Pingel JT, Thomas ML. Sequence conservation in potentially regulatory regions of the mouse and human leukocyte common antigen gene. J Biol Chem 1989;264:6220–6229.
4. Saga Y, Tung JS, Shen FW, Boyse EA. Alternative use of 5' exons in the specification of Ly-5 isoforms distinguishing hematopoietic cell lineages. Proc Natl Acad Sci USA 1987;84:5364–5368.
5. Streuli M, Hall LR, Saga Y, Schlossman SF, Saito H. Differential usage of three exons generates at least five different mRNAs encoding human leukocyte common antigens. J Exp Med 1987;166:1548–1566.
6. Charbonneau H, Tonks NK, Walsh KA, Fischer EH. The leucocyte common antigen (CD45): A putative receptor-linked protein tyrosine phosphatase. Proc Natl Acad Sci USA 1988;85:7182–7186.
7. Tonks NK, Charbonneau H, Diltz CD, Fischer EH, Walsh KA. Demonstration that the leukocyte common antigen CD45 is a protein tyrosine phosphatase. Biochemistry 1988;27:8695–8701.
8. Sanders ME, Makgoba MW, Shaw S. Human naive and memory T-cells: reinterpretation of helper–inducer and suppressor–inducer subsets. Immunol Today 1988;9:195–199.
9. Shah VO, Civin CI, Loken MR. Flow cytometric analysis of human bone marrow. IV. Differential quantitative expression of T-200 common leukocyte antigen during normal hemopoiesis. J Immunol 1988; 140:1861–1867.
10. Jensen GS, Poppema S, Mant MJ, Pilarski LM. Transition in CD45 isoform expression during differentiation of normal and abnormal B-cells. Int Immunol 1989;1:229–236.
11. Caldwell CW, Patterson WP, Toalson BD, Yesus YW. Surface and cytoplasmic expression of CD45 antigen isoforms in normal and malignant myeloid cell differentiation. Am J Clin Pathol 1991;95:180–187.
12. Master PS, Richards SJ, Roberts BE, Scott CS. Patterns of membrane CD45 isoform expression by leukaemic blasts and normal mature myeloid cells. Int J Hematol 1992;55:235–242.
13. Kawano S, Tatsumi E, Yoneda N, Yamaguchi N. Pattern of expression of CD45 RA/RO isoformic antigens in acute myeloblastic leukemia cells. Am J Clin Pathol 1993;100:386–393.
14. Schiavone, EM, Lo Pardo C, Di Noto R, Manzo C, Ferrara F, Vacca C, Del Vecchio L. Expression of the leucocyte common antigen (LCA, CD45) isoforms RA and RO in acute haematological malignancies: possible relevance in the definition of new overlap points between normal and leukaemic haemopoiesis. Br J Haematol 1995;91:899–906.
15. Falcao RP, Garcia AB. Expression of CD45RA (naive) and CD45RO (memory) antigens in T-acute lymphoblastic leukaemia. Br J Haematol 1993;85:483–486.
16. Dang AM, Phillips JA, Lin T, Raveche ES. Altered CD45 expression in malignant B-1 cells. Cell Immunol 1996;169:196–207.
17. Zola H, Siderius N, Flego L, Sparrow-R, van der Weyden MB, Nimmo J, Hart DN, Houghton S, Boyd AW. Expression of CD45 isoforms in chronic B-cell leukaemias. Leuk Res 1993;17:209–216.
18. Bennett JM, Catovsky D, Daniel M-T, Flandrin G, Galton DAG, Gralnick HR, Sultan C. Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French–American–British Cooperative Group. Ann Intern Med 1985;103:620–625.
19. Bennett JM, Catovsky D, Daniel M-T, Flandrin G, Galton DAG, Gralnick HR, Sultan C. Proposal for the recognition of minimally differentiated acute myeloid leukaemia (AML-M0). Br J Haematol 1991; 78:325–329.
20. Miyachi H, Takemura Y, Yonekura S, Komatsuda M, Nagao T, Arimori S, Ando Y. MDR1 (multidrug resistance) gene expression in adult acute leukemia: coorelations with blast phenotype. Int J Hematol 1993;57:31–37.
21. Catovsky D, Matutes E, Buccheri V, Shetty V, Hanslip J, Yoshida N, Morilla R. A classification of acute leukaemia for the 1990s. Ann Hematol 1991;62:16–21.
22. Buccheri V, Matutes E, Dyer MJS, Catovsky D. Lineage commitment in biphenotypic acute leukemia. Leukemia 1993;7:919–927.
23. Lansdorp PM, Sutherland HJ, Eaves CJ. Selective expression of CD45 isoforms on functional subpopulations of CD34+ hemopoietic cells from human bone marrow. J Exp Med 1990;172:363–366.
24. Fujii Y, Okumura M, Inada K, Nakahara K, Matsuda H. CD45 isoform expression during T-cell development in the thymus. Eur J Immunol 1992;22:1843–1850.